Comparative analytical study of active compounds from *Zingiber officinale*

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The selective alcoholic extraction, isolation and characterisation of active compounds from ginger were studied. The paper aims to investigate a comparative analytic study of two sample of ginger (fresh and dried sample) by chromatography, UV-VIS spectroscopy and scanning electronic microscopy.

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1. Introduction

Although worldwide there are many studies on natural products, research in this area continues to be of great potential due to the growing demand for valuable natural compounds. For centuries, ginger (*Zingiber officinale)* has been widely used as a dietary supplement, spice and a medicinal herb in traditional medicine. The studies regarding the ginger active compounds extraction and separation approaches are applied to assess the chemical and biological process engineering for obtaining some natural compounds valuble in different domains: medical, pharmaceutical, food industry. Gingerols and shagaols, the principal pungent components of *Zingiber officinale*, present antipyretic, antitussive, hypotensive, cardiotonic, anti-inflammatory, analgesic, cytotoxic, antitumor, antioxidant, antihepatotoxic, antifungal and antiemetic properties. (G.Singh, 2008; D.A Baladin, 1999; P.N Ravindaran, 2005; H.A. Schwertner, 2007; S.I.Al Qasoumi, 2009; K.C. Srivastava 1989; M. Thomson, 2002; J.P. Li, 2001; X.L.Yang, 1999; B.H. Ali, 2008).

Many analytical methods have been reported for ginger constituents from different types of solvents. (X. Li, 2008).

The use of solvents with relative high toxicity and the formation of deposits of hazardous waste represent the main disadvantages of the solvent extraction methods. Therefore, the objective of this investigation was to develop a simple, eco-friendly, economical and selective alcoholic extraction and characterisation of ginger active compounds. A series of aliphatic alcohols (methanol, ethanol and isopropanol) was used during the experimental determinations. The paper describes the metal content by atomic absorption spectroscopy, morphology and elemental composition by scanning electron microscopy and EDX to estimate the diversity of these two different samples of ginger (fresh and dried powder) with a comparative examination. This study focus on the comparison between fresh and dried gingers, and so their differences and similarity should be described, highlighted and discussed

2. Experiments

Material: ginger fresh rhizome and dried ginger powder.

The fresh rhizome material was grinded to 20 mesh or less.

The dried ginger sample was prepared by simple grinding and dry at the temperature of 80 ° C for 6h.

Methods

Extraction of Active Constituents. The sample of *Zingiber officinale*, 0.4 - 0.5 g (±0.1 mg), was placed into a 25-mL volumetric flask with 20 mL alcohol, then sonicated for 30 minutes at room temperature. After that, the sample solution is filtered through 0.45 µm.

GC-MS Analyses. The active constituents were analyzed by GC-MS. Analysis was carried out using an Sistem **G1701EA GC/MSD** Agilent 7890 N gas chromatograph equipped with a HP-5MS capillary column (5% phenylmethylsiloxane, 30 m x 250 µm, film thickness, 0.25 µm, Agilent Technologies, USA) coupled with a 5975B mass selective detector spectrometer from the same company. The front inlet was kept at 250°C in splitless mode. The temperature program was as follows: initial column temperature 100°C, held for 1 min, then programmed to 150°C at a rate of 10 °C/min and held for 5 min; 200°C at a rate of 10°C/min and held for 5 min; then programmed to 250°C at a rate of 5°C/min and held for 10 min; finally programming to 300°C at a rate of 5°C/min, then held at 300°C for 10 min. As a carrier gas, helium at 1.0 mL min^{-1} was used. MS conditions: the detector was used in the EI mode with an ionization voltage of 70 eV.

The ion source temperature was at 230°C. The transfer line was at 250°C. The spectra were collected at 3 scans/s over the mass range (*m*/*z*) 30–440.

- The volatile active constituents *(Table 1)* were identified by comparison of their linear retention indices (relative to C8-C26 alkanes on the HP-5MS column and their mass spectra with those standards from and NIST). The percentage composition of the volatiles active compounds volatile was computed from the GC peak areas normalization without any corrections.
- ***UV-VIS analysis.*** The spectrophotometric measurements were carried out on a LAMBDA 950, Perkin Elmer. The samples were properly diluted with different quantities of methanol (5-15 mL) and were analyzed in quartz cuvettes.
- ***HPLC-DAD Analysis*** The contents of active compounds from ginger samples were analyzed by high performance liquid chromatography (HPLC 3000, Ultimate, Germany) equipped with a column C18 (YMC-Pack™ ProC18™ Column - Hypersil∨ODS 250 mm x 4.0 mm i.d., 5 µm). Elution was isocratic using a mixture of HPLC grade acetonitrile and water (55:45 v/v) flow rate 1.0 ml/min, temperature 30_C. The DAD (diode array detection) was set at 425 nm (for signal A), 280 nm (for signal B), and 240 nm (for signal C), at 4 nm bandwidth individually, with 550 nm reference wavelength, at 50 nm bandwidth. Full spectral scanning was also performed from 200-600 nm, with range steps of 2 nm.

A gradient elution (0-8 min: 55% A - 45% B, 8.00-15.00 min:50 % A – 50% B; 15.00 - 40.00 min:45%A - 55%B; 40.00-45.00 min: 50 % A – 50% B; 45.00-55.00 min – 55 % A – 45% B 90%) was run with mobile phase solvents A (HPLC grade water**)** and B (HPLC grade acetonitrile) with a flow rate of 0.2 mL/min and injection volume of 10µL.

- ***Atomic absorption spectrometry (AAS)***

The tests for determination of heavy metals contents from ginger samples were conducted under international standard ISO 15586:2003 (E), on equipment: Analytik Jena novAA 400G - apparatus, with a graphite furnace, equipped with autosampler MPE60 and software WinAAS 3.17.0.

Examined materials: samples of ginger (0.2541 g) weighed on a Sartorius analytical balance, with an accuracy of ± 0.0001 g.; Substances: nitric acid, ultrapure water. The samples is treated with 5.5 mL HNO_3 65% and subjected to digestion in a Berghof microwave oven MWS 2, using a three stages program: T_1=160°C, t_1= 15 min, p_1= 80%. T_2=210 °C, t_2=15 min, p_2=90% si T_3 in scadere, t_3=15 min, p_3=0%. After digestion, the sample is brought to a volume of 100 mL with ultrapure water.

- ***Scanning electron microscopy (SEM/EDAX)***

To highlight the morphology and elemental composition of two types of ginger were analyzed by scanning electron microscopy (SEM) using Inspect S PANalytical model coupled with the energy dispersive X-ray analysis detector (EDX).

3. Results and disscution

It well known that ginger chemical composition depends on the harvesting area and rhizomes status (fresh or dry). It was demonstrate a slightly difference between the gingerols, shogaols and volatile oil concentration in fresh and dry ginger. (Ali, B.H, 2008; A. Ghasemzadeh, 2010; N. Pawar, 2011). The volatile oil of ginger is a mainly a mixture of monoterpenic and sesquiterpenic compounds, responsible for the characteristic ginger flavour and α zingiberene is the major component. In the dried ginger, the sesquiterpenes: β-sesquiphellandrene and zingiberene are converted to ar-curcumene. (W.K.S.M Abeysekera, 2005).

Ginger oil contains mainly zingiberene, bisaboline and other sesqui and monoterpenes. Ginger oleoresin contains especially the pungent principles gingerols and shogaols and also zingiberone.(M. Sultan, 2005; Z. Yang, 2009; I. A. Abu-Yousef, 2011).

- ***GC-MS Analysis***

The volatile compounds from the both ginger samples were identified by GC-MS chromatography. The results obtain conduct to identification of six different compounds from ginger samples (*Table 1*).

Table 1.

Retention time	Compounds identified from GC-MS library
7.908	α-curcumen
8.135	zinziberen
8.731	retinal
24.931	6-gingerol
48.24	sistasterol
53.75	Acid cinamic

- **UV-VIS analysis.** The wavelength identified by UV-VIS spectra are presented in *Table 2*.
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Table 2.

Sample	λmax (nm)
1	205, 230, 280, 370
2	230, 280, 365

- fresh sample alcoholic extract
- dried sample alcoholic extract

- ***HPLC analysis***

The HPLC method was used for a comparative investigation for the identification of ginger active compounds from three different alcoholic extracts. Results of HPLC-DAD analysis are presented in Tabel 3.

Table 3. HPLC-DAD results.

No	Retention time (mAU*min)	Area (mAU)	Height	Compounds identified
1	17.398	11.1725	19.648	gingerol
	53.093	10.036	41.006	Borneol
	55.730	15.907	58.724	curcumene
2	11.262	2.672	15.670	ND
	17.398	14.1725	16.632	gingerol
	52.996	8.054	33.266	Borneol
	55.643	14.905	53.795	curcumene
	58.622	6.560	23.077	zingiberene
3	17.398	11.1725	19.648	gingerol
	53.118	18.053	35.223	Borneol
	58.612	7,568	21.451	zingiberene

1. MeOH extract
2. EtOH extract
3. Isopropanol extract

Identification of compounds of the alcohol extracts chromatograms: *gingerol*, *zingiberene* (a monocyclic sesquiterpene, curcumene (sesqueterpenoid) and borneol (a terpene).

- ***Atomic absorption spectrometry analysis***

The actual state of atomic absorption spectrometry of two types of ginger as the method of determining As, Cu, Pb, Zn, Mn, Fe and Ni is described on the basis of literature data.

From the results obtained, it can be seen that metals such Cu, Fe were found both in *fresh rhizome* and *dried sample (Table 4)*.

Table 4. The metals content from the ginger samples.

No	Sample	As	Cu	Pb	Zn	Fe	Ni	Cd
1.	*Fresh Rhizome*	2.237	6.923	*	13.857	130.5	*	*
2.	*Dried sample*	*	53.261	*	*	205.5	2.429	*

* below the detection device

- ***Scanning electron microscopy (SEM/EDAX)***

To highlight the morphology and elemental composition, two types of ginger sample were analyzed by scanning electron microscopy coupled with EDAX. We made a comparative study between the *fresh rhizome* and the *dried ginger sample.* Thus, the topographic surface analysis shows that heat treatment as applied in the drying material did not cause changes in morphology. The results are shown in following figures.

From the SEM images (Fig. 1. (a)) the surface topography on the fresh ginger can be observed. This shows a fibrous structure with a thickness about few μm. EDAX analysis provided a semi quantitative elemental analysis of the surface indicating the elements of the study material.

From EDX spectrum analysis, it can be seen that both samples have approximately the same elemental composition, which means dried ginger sample has not changed elemental composition during the heat treatment applied.

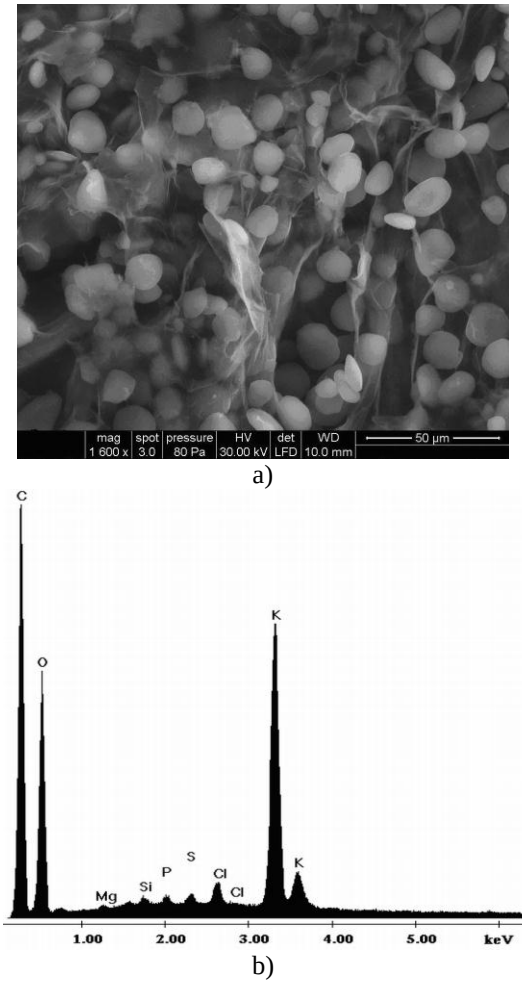
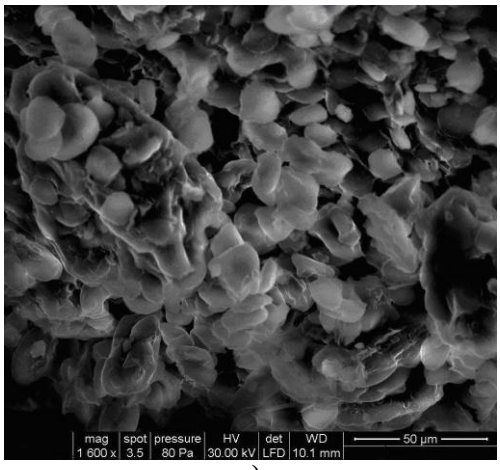
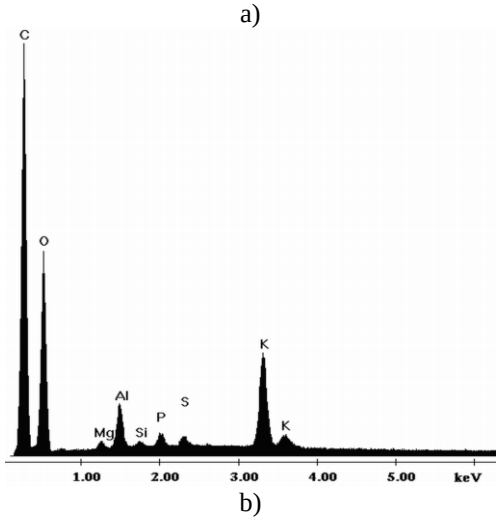


Fig. 1. SEM morphology (a) and EDX elemental analysis (b) for fresh ginger.



a)



b)

Fig. 2. SEM morphology (a) and EDX elemental analysis (b) for dried ginger.

4. Conclusions

The extraction of both ginger samples was made in alcohol and hexan. GC-MS chromatography was used for identifying and separation of compounds from fresh and dried samples: α–curcumene, zingiberen, retinal; 6–gingerol; sitasterol, etc. UV-Vis spectroscopy facilitates the HPLC analysis. HPLC –DAD chromatography allows the separation of gingerol from both samples.

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